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13. ABSTRACT *(Maximum 200 Words)*

Flavopiridol is an investigational drug undergoing Phase II clinical trials for the treatment of various solid tumors. This drug has been shown to inhibit of the cyclin-dependent protein kinases, which are essential mediators of the cell division cycle. While this activity is likely to be responsible for its anticancer activity, it is nevertheless unclear how this drug achieves selective toxicity. To address this question, we created a flavopiridol-resistant cell line. The experiments described in this proposal are designed to identify the mechanism(s) responsible for rendering this cell line drug-resistant. Levels of cyclin-dependent protein kinase activity in this resistant cell line will be determined, and compared to those seen in the parental, drug-sensitive cell. In addition, levels of relevant drug-detoxifying enzymes, such as UDP-glucoronosyl transferase and glutathione S-transferase will be measured in these two cell lines, as will levels of drug transporters. Additional strategies will examine whether other gene products are over or under expressed in drug-resistant cells, as well. It is anticipated that the results of these studies will shed light on the mechanism of flavopiridol resistance. This information could be of value in designing second generation drugs that may prove more effective in the treatment of cancer.

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Introduction

The objective of this proposal is to gain insight into the molecular mechanism of cellular sensitivity to Flavopiridol (FP), an anticancer agent. FP is an investigational new drug currently in Phase II clinical trials for the treatment of solid tumors. The precise mechanism of action of this compound is unknown. It is also unclear why this compound appears to preferentially target some cancer cells, while sparing normal cells. A FP-resistant human breast adenocarcinoma cell line, named MCF-7/FP, will be used to address these issues. Specifically, the experiments described in this proposal are designed to identify the molecular basis for FP resistance in MCF-7/FP cells. Cellular levels of known FP targets, i.e. cyclin-dependent protein kinases will be measured in these cells. In addition, the relative resistance of these cells to FP analogues, will be determined. It is anticipated that FP or FP-like molecules will ultimately assume a place in the modern cancer chemotherapeutic armamentarium. Thus, insight gained into the molecular basis of FP drug resistance in MCF-7/FP cells may ultimately prove beneficial in the design of second or third generation FP analogues. It is also conceivable that this information may aid in the development of chemotherapy strategies to minimize the emergence of clinical resistance to these agents.

Body

On 27 July, 2000 I requested to be named as a replacement Principal Investigator for Army Grant No. DAMD17-99-1-9299. I was informed on 15 September, 2000, that my request had received favorable consideration. Thus, we have not yet made significant progress towards any of the Tasks outlined in the approved statement of work. However, the following section briefly outlines our current efforts towards these objectives.

Task 1. Ascertain whether human normal breast epithelial MCF-10A cells are, relative to human breast adenocarcinoma MCF-7/0 cells, less sensitive to FP.

Preliminary results relayed to me by Dr. Normal Sladek, the original PI of this proposal indicate that, contrary to what was anticipated, the MCF-10A cells line is actually several fold more sensitive to the cytotoxic effects of FP than are MCF-7/0 cells (data not shown). We are in the process of repeating these experiments, but have yet to obtain any data.

Task 2. Ascertain whether stable resistance to FP on the part of MCF-7/FP cells persists beyond 90 cell divisions.

We are in the process now of growing the MCF-7/FP cells in both the presence and absence of FP for the requisite period of time to address this experiment. Sufficient time has not passed for these efforts to yield results.

Task 3. Ascertain whether the sensitive MCF-7/0 cell line and the insensitive MCF-7/FP subline differ in selected cell cycle parameters.

These experiments have not yet been initiated. We anticipate doing so in the next several months

Task 4. Ascertain whether MCF7/FP cells are cross-resistant to flavopiridol analogues, other flavone anticancer agents, UCN-01, and/or anticancer agents presently used to treat metastatic breast cancer.

These experiments have not yet been initiated. We anticipate that these experiments will be initiated within the next several weeks, and will require approximately 10-18 months to complete.

Task 5. Ascertain whether elevated levels of the kinase(s) innhibited by FP account for MCF-7/FP insensitivity to this agent.

We have obtained antibodies specific for several cyclin-dependent protein kinases, and will be addressing this question in the coming months. Preliminary efforts currently underway are focusing on measuring the levels of these proteins in extracts from MCF-7/0 cells. PCR experiments designed to amplify probes appropriate for use in northern blot hybridizations are also underway. We anticipate initiating the northern blot experiments within 2-3 months.

Tasks 6 and 7. As was outlined in the original proposal, these tasks will only be pursued if completion of Tasks 5 and 6 do not provide a full phenotypic explanation for the acquired resistance of the MCF-7/FP cells. Thus, we do not anticipate addressing these objectives within the next 12 months (if at all).

Key Research Accomplishments. None

Reportable Outcomes. None

Conclusions. Insufficient time has elapsed since notification of receipt of this award for any meaningful results to have been achieved. However, in the last 6 weeks, we have gained valuable experience in handling the MCF-7-derived cell lines. We are confident

that these initial efforts have laid the foundation for more significant research accomplishments in the coming months.

References. None